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(54) Title: ENZYMATIC DETERGENT COMPOSITIONS			
(57) Abstract			
<p>Enzymatic detergent compositions comprise mutant subtilisin protease, in certain detergent compositions formulated as detergent powders containing phosphate or zeolite builder, aqueous detergent liquids, nonsqueeguous detergent liquids, or detergent bars.</p>			

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Enzymatic Detergent Compositions

25 This invention relates to enzymatic detergent compositions, and in particular to compositions containing mutant subtilisin proteases. Prior-filed International Patent Application WO 89/06279 (not however prior-published) discloses certain mutant subtilisin-type proteases, and their use in washing compositions. The present invention relates in particular to novel detergent compositions incorporating mutant proteases as described in Application WO 89/06279.

30 In particular embodiments the invention further relates to the use of the modified proteases in conjunction with lipases.

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Prior Art:

Subtilisin proteases and detergent compositions containing them, especially laundry detergents, are well known and have been widely used. Lipases are also known and used as ingredients of detergent and cleaning compositions.

A number of patent specifications and other documents describe various mutant hydrolytic enzymes including 10 subtilisin proteases, some of which are expressed to be suitable for detergent or cleaning applications: Examples include EP 0 130 756 (Genentech), EP 0 214 435 (Henkel), WO 87/04461 (Amgen), WO 87/05050 (Genex), EP 87303761 (Genentech), EP 0 260 105 (Genencor), WO 88/08028 (Genex), 15 WO 88/06624 (Gist-Brocades), WO 88/07578 (Genentech) and WO 88/08033 (Amgen). Thomas et al (in Nature, 318 (1985) 375-6) have described a mutation in subtilisin protease EPN' that changes the pH dependence of the enzyme.

20 Prior-filed International Patent Application WO 89/06279 (not however prior-published) discloses the mutant subtilisin-type proteases which are applied in the present application, and describes tests of them under certain washing conditions.

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The present invention:

According to the present invention there is provided an enzymatic detergent composition, comprising a mutated 30 subtilisin protease, characterised in that said protease contains, relative to the corresponding parent protease, an amino-acid residue at one or more of the following positions which has been changed by substitution, insertion or deletion:

- 3 -

6,9,11,12,19,25,36,37,38,53,54,55,56,57,58,59,67,71,89,
111,115,120,121,122,124,128,131,140,153,154,156,158,159,
160,161,162,163,164,165,166,168,170,172,175,180,182,186,
187,191,194,195,199,218,219,226,234,235,236,237,238,241,
5 260,261,262,265,268,275,

and in that the remainder of the detergent composition is either:

10 (a) formulated as a detergent powder containing phosphate builder, anionic surfactant, nonionic surfactant, acrylic or equivalent polymer, perborate bleach precursor, amino-containing bleach activator, silicate or other structurant, alkali to adjust to desired pH in use, and neutral inorganic salt; or

15 (b) formulated as a detergent powder containing zeolite builder, anionic surfactant, nonionic surfactant, acrylic or equivalent polymer, perborate bleach precursor, amino-containing bleach activator, silicate or other structurant, alkali to adjust to desired pH in use, and neutral inorganic salt; or

20 (c) formulated as an aqueous detergent liquid comprising anionic surfactant, nonionic surfactant, humectant, organic acid, caustic alkali, with a pH adjusted to a value between 9 and 10; or

25 (d) formulated as a nonaqueous detergent liquid comprising a liquid nonionic surfactant consisting essentially of linear alkoxylated primary alcohol, triacetin, sodium tripolyphosphate, caustic alkali, perborate monohydrate bleach precursor, and tertiary amine bleach activator, with a pH adjusted to a value between about 9 and 10; or

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5 (e) formulated as a detergent powder in the form of a granulate having a bulk density of at least 600 g/l, containing anionic surfactant and a mixture of nonionic surfactants with respective alkoxylation degrees about 7 and about 3, low or substantially zero neutral inorganic salt, phosphate builder, perborate bleach precursor, tertiary amine bleach activator, sodium silicate, and minors and moisture; or

10 (f) formulated as a detergent powder in the form of a granulate having a bulk density of at least 600 g/l, containing anionic surfactant and a mixture of nonionic surfactants with respective alkoxylation degrees about 7 and about 3, low or substantially zero neutral inorganic salt, zeolite builder, perborate bleach precursor, tertiary amine bleach activator, sodium silicate, and minors and moisture; or

15 (g) formulated as a detergent powder containing anionic surfactant, nonionic surfactant, acrylic polymer, fatty acid soap, sodium carbonate, sodium sulphate, clay particles, perborate bleach precursor, tertiary amine bleach activator, sodium silicate, and minors and moisture; or

20 (h) formulated as a detergent (soap) bar containing soap based on pan-saponified mixture of tallow and coconut oil, neutralised with orthophosphoric acid, mixed with protease, also mixed with sodium formate, borax, propylene glycol and sodium sulphate, and then plodded on a soap production line.

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30 Detergent compositions according to the present invention can give advantages including improved performance and/or enzyme stability as compared with known compositions.

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References herein to numbered aminoacid sequence positions which are the subject of mutations in subtilisin proteases to be used in accordance with this invention, are references to aminoacid residues and their number as they occur in the following sequence, which is that of subtilisin BPN'. The invention also includes modified variants of other subtilisin proteases, and in order to apply the numbered mutation sites referred to herein to such another protease, the numerical part of the reference 5 is to be understood as a reference to the corresponding position of such other homologous subtilisin protease in the sense of its maximum homology with subtilisin BPN'. Such a corresponding position may differ in number along the chain of the other protease by reason of apparent 10 deletion(s) or insertion(s) in the gene of such other protease by comparison with that of the gene of BPN' or other reference sequence. A deletion or absent aminoacid 15 is indicated by **, and an insertion relative to BPN' by a lower case alphabetical suffix on the position number.

Subtilisin BPN' has the following sequence (ref Wells et al 20 (1983) Nucleic Acids Res. 11, 7911-7925):

No:	1	10
25	*-*-*-*-*-*-*A-Q-S-*V-P-Y-G-V-S-Q-I-K-*-*-*-*-*A-p-A-	
No:	20	30
30	L-H-S-Q-G-Y-T-G-S-N-V-K-V-A-V-I-D-S-G-I-D-S-S-H-P-D-L-**	
No:	50	60
35	*-K-V-A-G-G-A-S-M-V-P-S-E-T-N-P-F-**-Q-D-N-N-S-H-G-T-H-V-	
No:	70	80
40	A-G-T-V-A-A-L-*N-N-S-I-G-V-L-G-V-A-P-S-A-S-L-Y-A-V-K-V-	
No:	100	110
45	L-G-A-D-G-S-G-Q-Y-S-W-I-I-N-G-I-E-W-**-A-I-A-*N-N-M-D-**	
No:	130	140

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*****-V-I-N-M-S-L-G-G-P-S-G-S-A-A-L-K-A-A-V-D-K-A-V-A-

No:	150	160	170	
	S-G-V-V-V-A-A-G-N-E-G-T-S-G-S-S-S-T-V-G-Y-P-G-K-Y-P-			
No:	180	190	200	
5	S-V-I-A-V-G-A-V-D-S-S-N-Q-R-A-S-F-S-S-V-G-P-E-L-D-V-M-A-			
No:	210	220		
	P-G-V-S-I-Q-S-T-L-P-G-N-*-*K-*-*Y-G-A-Y-N-G-T-S-M-A-S-P-H-			
No:	230	240	250	
	V-A-G-A-A-A-L-I-L-S-K-H-P-N-W-T-N-T-Q-V-R-S-S-L-E-N-T-T-			
10	No: 260	270	275	
	T-K-L-G-D-S-F-Y-Y-*-*G-K-G-L-I-N-V-Q-A-A-A-Q			

Subtilisin 309 has the following sequence:

(ref Patent Application PCT/DK 88/00002=WO 89/06279)

15	No: 1	10	
	*****-A-Q-S-*-*V-P-W-G-I-S-R-V-Q-*-*-*-*-*A-P-A-		
No:	20	30	40
	A-H-N-R-G-L-T-G-S-G-V-K-V-A-V-L-D-T-G-I-*-*S-T-H-P-D-L-*-		
20	No: 50	60	
	-N-I-R-G-G-A-S-F-V-P-G-E-P--*S-T-*-*Q-D-G-N-G-H-G-T-H-V-		
No:	70	80	90
	A-G-T-I-A-A-L-*-*N-N-S-I-G-V-L-G-V-A-P-S-A-E-L-Y-A-V-K-V-		
25	No: 100	110	120
	L-G-A-S-G-S-G-S-V-S-S-I-A-Q-G-L-E-W-*-*A-G-N-*-*N-G-M-H-*-		
No:	130	140	
	--*-*V-A-N-L-S-L-G-S-P-S-P-S-A-T-L-E-Q-A-V-N-S-A-T-S-		
No:	150	160	170
	R-G-V-L-V-V-A-A-S-G-N-S-G-A-*-*G-S-I-S-*-*-*Y-P-A-R-Y-A-		
30	No: 180	190	200
	N-A-M-A-V-G-A-T-D-Q-N-N-N-R-A-S-F-S-Q-Y-G-A-G-L-D-I-V-A-		
No:	210	220	
	P-G-V-N-V-Q-S-T-Y-P-G-S-*-*T-*-*Y-A-S-L-N-G-T-S-M-A-T-P-H-		
35	No: 230	240	250

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V-A-G-A-A-A-L-V-K-Q-K-N-P-S-W-S-N-V-Q-I-R-N-H-L-K-N-T-A-

No: 260 270 275

T-S-L-G-S-T-N-L-Y-*G-S-G-L-V-N-A-E-A-A-T-R

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In the above sequences the individual letter codes indicate amino-acid residues in accordance with the following abbreviations:

10	A =	Ala =	Alanine
	V =	Val =	Valine
	L =	Leu =	Leucine
	I =	Ile =	Isoleucine
	P =	Pro =	Proline
15	F =	Phe =	Phenylalanine
	W =	Trp =	Tryptophan
	M =	Met =	Methionine
	G =	Gly =	Glycine
	S =	Ser =	Serine
20	T =	Thr =	Threonine
	C =	Cys =	Cysteine
	Y =	Tyr =	Tyrosine
	N =	Asn =	Asparagine
	Q =	Gln =	Glutamine
25	D =	Asp =	Aspartic acid
	E =	Glu =	Glutamic acid
	K =	Lys =	Lysine
	R =	Arg =	Arginine
30	H =	His =	Histidine

It has also been surprisingly found that compatibility of protease with lipase is influenced by the pI of the protease and by the positions at which the charges are located relative to the active site of the protease: the

introduction of negative charge or removal of positive charge to give a decrease in the isoelectric point pI, can improve compatibility of protease with lipase, and making the mutations of this kind closer to the active site can give stronger improvement of compatibility of protease with lipase.

The isoelectric point, pI, is the pH value where the enzyme molecule (with optionally attached metal or other ions) is neutral, i. e. the sum of electrostatic charges is zero.

The isoelectric point is conveniently calculated (approximately) by using pK values for the various charged residues in the enzyme in question and then by iteration find the pH value where the net charge of the enzyme is zero.

The pI can also be determined experimentally by isoelectric focussing or by titrating a solution containing the enzyme. The various pK values for the charged residues may also be determined by titration.

(Mutant proteases with modified pI are described in copending application _____ (claiming priority from UK 8914604.7 of 26/6/89), which describes and claims detergent compositions containing them, and the corresponding mutant proteases themselves are described and claimed in copending application _____ (claiming priority from DK 3169/89 of 26/6/89).)

In certain embodiments of the detergent compositions the mutated subtilisin protease contains, relative to the corresponding parent protease, an amino-acid residue at within about 20A of the active site which has been changed

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by substitution, deletion, or adjacent insertion, e.g. at one or more of positions 6, 36-38, 53-59, 89, 128, 131, 156, 158-160, 162-164, 166, 170, 182, 186, 195, and/or 218.

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Detailed information about the structure and aminoacid numbering sequence of the subtilisin proteases, are given in prior-filed International Patent Application WO 89/06279, and in copending application _____
10 (claiming priority from UK Patent Application 8914604.7 of 26/6/89), both incorporated herein by reference.

Techniques for the production of the mutant proteases are described in WO 89/06279 (hereby incorporated herein by 15 reference), and further applicable such methods are also given in the above-cited copending application (also incorporated herein by reference).

In particular detergent compositions within the scope of 20 the invention, the protease can represent a mutation of a parent enzyme selected from subtilisin RPN', subtilisin amylo-saccharolyticus, subtilisin 168, subtilisin mesinticopeptidase, subtilisin Carlsberg, subtilisin DV, subtilisin 309, subtilisin 147, subtilisin thermitase, and proteinase K, or aqualysin.

In particular examples, the protease can represent a 25 mutation of a parent enzyme altered to include any of the following amino-acid residues indicated by their abbreviations and located at the indicated sequence position: any of the mutations 6-tyr, 67-glu, 67-asp, 68-cys, 68-met, 71-glu, 168-ala, 170-tyr, 175-ile, 219-met, 275-glu, 19-gly+219-cys, 153-ala+218-ser.

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In further examples, the protease mutation can consist of an insertion of one or more aminoacid residues at any of positions 36, 56, 159, 164-166.

5 Specific mutant examples preferred for use in connection with this invention are as follows and referred to by the following labels:

S1 = G195E;
10 S3 = R170Y;
S4 = G195E+R170Y;
S12 = G195E+R170Y+K251E;
S19 = G195E+R170Y+H120D+K235L;
S20 = G195E+R170Y+H120D+K235L+K251E;
15 C2 = D120K;
C3 = D140K;
C4 = D120K+D14K;
C6 = D120K+K27D;
C8 = D172K;
20 C10 = D14K+D120K+D140K+D172K.

The S series mutants are preferably based on subtilisin 309 as the parent subtilisin. The C mutants are preferably based on subtilisin BPN'/subtilisin Carlsberg.
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In particular embodiments the invention presents enzymatic detergents comprising the proteases defined herein and with the features:

30 An enzymatic detergent composition formulated to give a wash liquor pH of 9 or less when used at a rate corresponding to 0.4-0.8 g/l surfactant.

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An enzymatic detergent composition formulated to give a wash liquor pH of 8.5 or more when used at a rate corresponding to 0.4-0.8 g/l surfactant.

- 5 An enzymatic detergent composition formulated to give a wash liquor ionic strength of 0.03 or less, e.g. 0.02 or less, e.g. 0.01 or less, when used at a rate corresponding to 0.4-0.8 g/l surfactant.
- 10 An enzymatic detergent composition formulated to give a wash liquor ionic strength of 0.01 or more, e.g. 0.02 or more, when used at a rate corresponding to 0.4-0.8 g/l surfactant.
- 15 The invention is illustrated by way of the following non-limitative Examples:

Example 1:

- 20 A detergent powder according to an embodiment of the invention containing phosphate builder is formulated to contain: total active detergent about 16%, anionic detergent about 9%, nonionic detergent about 6%, phosphate-containing builder about 20%, acrylic or equivalent polymer about 3.5%, perborate bleach precursor about 6-18%, amino-containing bleach activator about 2%, silicate or other structurant about 3.5%, enzyme about 8 glycine units/mg, with alkali to adjust to desired pH in use, and neutral inorganic salt, and enzymes (about 0.5% each enzyme).
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- 30

The anionic detergent is a mixture of sodium dodecylbenzene sulphonate 6% and primary alkyl sulphate 3%. The nonionic detergent is an ethoxylate of an approx. C13-C15 primary alcohol with 7 ethoxylate residues per

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5 mole. The phosphate builder is sodium tripolyphosphate. The polymer is polyacrylic acid. The perborate bleach precursor is sodium tetraborate tetrahydrate or monohydrate. The activator is tetraacetyl-ethylenediamine. The structurant is sodium silicate. The neutral inorganic salt is sodium sulphate. The enzyme is a protease according to Example S1. In alternative versions of this Example, the enzyme is selected from S3, S4, C2, C3, or C4.

10

Example 2:

15 A detergent powder according to an embodiment of the invention containing zeolite builder is formulated to contain: total active detergent about 16%, anionic detergent about 9%, nonionic detergent about 6%, zeolite-containing builder about 20%, acrylic or equivalent polymer about 3.5%, perborate bleach precursor about 6-18%, amino-containing bleach activator about 2%, silicate or other structurant about 3.5%, enzyme about 8 glycine units/mg, with alkali to adjust to desired pH in use, and neutral inorganic salt, and enzymes (about 0.5% each enzyme).

20

25 The anionic detergent is a mixture of sodium dodecylbenzene sulphonate 6% and primary alkyl sulphate 3%. The nonionic detergent is an ethoxylate of an approx. C13-C15 primary alcohol with 7 ethoxylate residues per mole. The zeolite builder is type A zeolite. The polymer is polyacrylic acid. The perborate bleach precursor is sodium tetraborate tetrahydrate or monohydrate. The activator is tetraacetyl-ethylenediamine. The structurant is sodium silicate. The neutral inorganic salt is sodium sulphate. The enzyme is a protease according to Example

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S1. In alternative versions of this Example, the enzyme is selected from S3, S4, C2, C3, or C4.

5 Example 3:

An aqueous detergent liquid according to an embodiment of the invention is formulated to contain:
10 Dodecylbenzene-sulphonic acid 16%, C12-C15 linear alcohol condensed with 7 mol/mol ethylene oxide 7%,
monoethanolamine 2%, citric acid 6.5%, sodium
xylenesulphonate 6%, sodium hydroxide about 4.1%, protease
0.5%, minors and water to 100%. The pH is adjusted to a
15 value between 9 and 10. The enzyme is a protease according
to Example S20. In alternative versions of this Example,
the enzyme is selected from S19, S12, S1, S3 or S4.

20 Example 4:

A nonaqueous detergent liquid according to an embodiment of the invention is formulated using 38.5% C13-C15 linear primary alcohol alkoxylated with 4.9 mol/mol ethylene oxide and 2.7 mol/mol propylene oxide, 5% triacetin, 30% sodium tripophosphate, 4% soda ash, 15.5% sodium perborate monhydrate containing a minor proportion of oxoborate, 4% TAED, 0.25% EDTA of which 0.1% as phosphonic acid, Aerosil 0.6%, SCMC 1%, and 0.6% protease. The pH is adjusted to a
25 value between 9 and 10, e.g. about 9.8. The enzyme is a
protease according to Example S1, S3 or S4.
30

Example 5:

A detergent powder according to an embodiment of the invention is formulated in the form of a granulate having a bulk density of at least 600 g/l, containing about 20% by weight surfactant of which about 10% is sodium dodecylbenzene sulphonate, and the remainder is a mixture of Synperonic A7 and Synperonic A3 (about 5.5% to 4.5%), and zero neutral inorganic salt (e.g. sodium sulphate), plus phosphate builder about 33%, sodium perborate tetrahydrate about 16%, TAED activator about 4.5%, sodium silicate about 6%, and minors including sodium carbonate about 2%, and moisture content about 10%. Enzymes (about 0.5% each enzyme) are included. The protease enzyme is a protease according to Example S1. In alternative versions of this Example, the enzyme is selected from S3, S4, C2, C3, or C4.

Example 6:

A detergent powder according to an embodiment of the invention is formulated in the form of a granulate having a bulk density of at least 600 g/l, containing about 20% by weight surfactant of which about 9% is sodium dodecylbenzene sulphonate, and the remainder is a mixture of Synperonic A7 and Synperonic A3 (respectively about 5% & 6%), and zero neutral inorganic salt (e.g. sodium sulphate), plus zeolite builder about 30%, sodium perborate tetrahydrate about 14%, TAED activator about 3.6%, and minors including sodium carbonate about 9%, Deguest 2047 (TM) about 0.7%, and moisture content about 10%. Enzymes (about 0.5% each enzyme) are included. The protease enzyme is a protease according to Example S1. In alternative versions of this Example, the enzyme is selected from S3, S4, C2, C3, or C4.

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Example 7:

A detergent powder according to an embodiment of the invention is formulated to contain:

5 Dodecylbenzenesulphonic acid 6%, C12-C15 linear alcohol condensed with 7 mol/mol ethylene oxide 5%, fatty acid soap 3%, Sokolan CP5 polymer (TM) 3%, zeolite A 22%, sodium carbonate 10%, sodium sulphate 17%, clay particles 8%, sodium perborate tetrahydrate 13%, tetraacetyl-
10 ethylenediamine 2%, protease 0.5%, minors and water to 100%. The pH is adjusted to a value between 9 and 10. The enzyme is a protease according to Example S20. In alternative versions of this Example, the enzyme is selected from S19, S12, S4, S1, or S3.

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Example 8:

A detergent (soap) bar according to an embodiment of the invention is formulated as follows: soap based on pan-saponified 82% tallow, 18% coconut oil, neutralised with 0.15% orthophosphoric acid, mixed with protease (about 8 GU/mg of the bar composition) and mixed with sodium formate 2%, borax 2%, propylene glycol 2% and sodium sulphate 1%, is then plodded on a soap production line.
20
25 The protease enzyme is a protease according to Example S1. In alternative versions of this Example, the enzyme is selected from S3, S4, C2, C3, or C4.

30 In further embodiments of the invention, structured liquid detergents can for example contain, in addition to a protease as described herein, 2-15% nonionic surfactant, 5-40% total surfactant, comprising nonionic and optionally anionic surfactant, 5-35% phosphate-containing or non-phosphate-containing builder, 0.2-0.8% polymeric
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thickener, e.g. cross-linked acrylic polymer with m.w. over 10^6 , at least 10% sodium silicate, e.g. as neutral waterglass, alkali (e.g. potassium-containing alkali) to adjust to desired pH, preferably in the range 9-10 or upwards, e.g. above pH 11, with a ratio sodium cation: silicate anion (as free silica) (by weight) less than 0.7:1, and viscosity of 0.3-30 Pa.s (at 20 deg. C and 20 reciprocal secs).

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10 For example such detergents can contain about 5% nonionic surfactant C13-15 alcohol alkoxylated with about 5 EO groups per mole and with about 2.7 PO groups per mole, 15-23% neutral waterglass with 3.5 weight ratio between silica and sodium oxide, 13-19% KDH, 8-23% STPP, 0-11% sodium carbonate, 0.5% Carbopol 941 (TM). Protease may be incorporated at for example 0.5% of example S1.

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The invention is susceptible of modifications and variations of the present disclosure which extends to all 20 combinations and subcombinations of the features mentioned and described herein, in the following claims and in the specifications referred to herein and incorporated by reference.

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CLAIMS:

1: An enzymatic detergent composition, comprising a mutated subtilisin protease, characterised in that said protease contains, relative to the corresponding parent protease, an amino-acid residue at one or more of the following positions which has been changed by substitution, insertion or deletion:

10 6, 9, 11, 12, 19, 25, 36, 37, 38, 53, 54, 55, 56, 57, 58, 59, 67, 71, 89,
 111, 115, 120, 121, 122, 124, 128, 131, 140, 153, 154, 156, 158, 159,
 160, 161, 162, 163, 164, 165, 166, 168, 170, 172, 175, 180, 182, 186,
 187, 191, 194, 195, 199, 218, 219, 226, 234, 235, 236, 237, 238, 241,
 260, 261, 262, 265, 268, 275,

15 and in that the remainder of the detergent composition is either:

20 (a) a detergent powder containing phosphate builder, anionic surfactant, nonionic surfactant, acrylic or equivalent polymer, perborate bleach precursor, amino-containing bleach activator, silicate or other structurant, alkali to adjust to desired pH in use, and neutral inorganic salt; or

25 (b) a detergent powder containing zeolite builder, anionic surfactant, nonionic surfactant, acrylic or equivalent polymer, perborate bleach precursor, amino-containing bleach activator, silicate or other structurant, alkali to adjust to desired pH in use, and neutral inorganic salt; or

30 (c) an aqueous detergent liquid comprising anionic surfactant, nonionic surfactant, humectant, organic acid,

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caustic alkali, with a pH adjusted to a value between 9 and 10; or

5 (d) a nonaqueous detergent liquid comprising a liquid nonionic surfactant consisting essentially of linear alkoxylated primary alcohol, triacetin, sodium tripolyphosphate, caustic alkali, perborate monohydrate bleach precursor, and tertiary amine bleach activator, with a pH adjusted to a value between about 9 and 10; or

10 (e) a detergent powder in the form of a granulate having a bulk density of at least 600 g/l, containing anionic surfactant and a mixture of nonionic surfactants with respective alkoxylation degrees about 7 and about 3, low or substantially zero neutral inorganic salt, phosphate builder, perborate bleach precursor, tertiary amine bleach activator, sodium silicate, and minors and moisture; or

15 (f) a detergent powder in the form of a granulate having a bulk density of at least 600 g/l, containing anionic surfactant and a mixture of nonionic surfactants with respective alkoxylation degrees about 7 and about 3, low or substantially zero neutral inorganic salt, zeolite builder, perborate bleach precursor, tertiary amine bleach activator, sodium silicate, and minors and moisture; or

20 (g) a detergent powder containing anionic surfactant, nonionic surfactant, acrylic polymer, fatty acid soap, sodium carbonate, sodium sulphate, clay particles, perborate bleach precursor, tertiary amine bleach activator, sodium silicate, and minors and moisture; or

25 (h) a detergent (soap) bar containing soap based on pan-saponified mixture of tallow and coconut oil, neutralised with orthophosphoric acid, mixed with protease, also mixed

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with sodium formate, borax, propylene glycol and sodium sulphate, and then plodded on a soap production line.

5 2: A detergent composition as claimed in claim 1, further characterised in that the protease represents a mutation of a parent enzyme selected from subtilisin BPN', subtilisin amylasaccharolyticus, subtilisin 168, subtilisin mesinticopeptidase, subtilisin Carlsberg, 10 subtilisin DV, subtilisin 309, subtilisin 147, subtilisin thermitase, and proteinase K or aqualysins.

15 3: A detergent composition as claimed in any preceding claim, further characterised in that the protease is based on subtilisin 309 as the parent subtilisin.

20 4: A detergent composition as claimed in any preceding claim, further characterised in that the protease is based on subtilisin 147 as the parent subtilisin.

25 5: A detergent composition as claimed in any preceding claim, further characterised in that the protease contains one or more of the following mutations:

25 (a) insertion of one or more aminoacid residues at any of positions 36,56,159,164-166; or

30 (b) any of the mutations 6-tyr, 67-glu, 67-asp, 68-cys, 68-met, 71-glu, 168-ala, 170-tyr, 175-ile, 219-met, 275-glu, 19-gly+219-cys, 153-ala+218-ser.

35 6: An enzymatic detergent composition, as claimed in claim 1, characterised in that the detergent composition is formulated to give a wash liquor pH of 9 or less when used at a rate corresponding to 0.4-0.8 g/l surfactant.

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7: An enzymatic detergent composition, as claimed in claim 1, characterised in that the detergent composition is formulated to give a wash liquor ionic strength of 0.03 or less, e.g. 0.02 or less, e.g. 0.01 or less, when used at a rate corresponding to 0.4-0.8 g/l surfactant.

8: An enzymatic detergent composition, as claimed in claim 1, characterised in that the detergent composition is formulated to give a wash liquor pH of 8.5 or more when used at a rate corresponding to 0.4-0.8 g/l surfactant.

9: An enzymatic detergent composition, as claimed in claim 1, characterised in that the detergent composition is formulated to give a wash liquor ionic strength of 0.01 or more, e.g. 0.02 or more, when used at a rate corresponding to 0.4-0.8 g/l surfactant.

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INTERNATIONAL SEARCH REPORT

International Application No. PCT/GB 90/00986

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)^a

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC⁵: C 11 D 3/386, C 12 N 9/54

II. FIELDS SEARCHED

Minimum Documentation Searched^c

Classification System	Classification Symbols
IPC ⁵	C 12 N, C 11 D

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched^b

III. DOCUMENTS CONSIDERED TO BE RELEVANT^d

Category ^e	Citation of Document, ^f with indication, where appropriate, of the relevant passages ^g	Relevant to Claim No. ^h
Y	EP, A, 0251446 (GENENTECH, INC.) 7 January 1988 see claims; page 24, table 1 (cited in the application) --	1-9
Y	WO, A, 88/08028 (GENEX CORP.) 20 October 1988 see claims; page 21, line 10 - page 22, line 12 (cited in the application) --	1-9
P,Y	WO, A, 89/06279 (NOVO INDUSTRI A/S) 13 July 1989 see claims; page 10, lines 16-27; page 42, lines 20-28 (cited in the application) --	1-9
Y	Nature, volume 328, 6 August 1987, (London, GB),	1-9 . / .

^a Special categories of cited documents: "A"

"A" document defining the general state of the art which is not considered to be of particular relevance

"B" earlier document but published on or after the international filing date

"L" document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another document or for other special reasons (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

^b "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention^c "X" document of particular relevance; the claimed invention cannot be considered to involve an inventive step if the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.^d "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.^e "A" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

24th September 1990

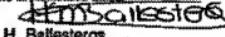
Date of Mailing of this International Search Report

17.10.90

International Searching Authority

EUROPEAN PATENT OFFICE

Signature of Authorized Officer


H. Ballisteros

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages	Relevant to Claim No.
	A.J. Russell et al.: "Rational modification of enzyme catalysis by engineering surface charge", pages 496-500 see the whole article (cited in the application) -----	

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.**

GB 9000986
SA 37858

This annex lists the patent family members relating to the patent document cited in the above-mentioned international search report. The numbers are as contained in the European Patent Office EPO file on 09/10/90. The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A- 0251446	07-01-88	AU-A-	7228187	05-11-87
		JP-A-	1085075	30-03-89
WO-A- 8808028	20-10-88	EP-A-	0353250	07-02-90
WO-A- 8906279	13-07-89	None		